Enhancing single-molecule photostability by optical feedback from quantum jump detection

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We report an optical technique that yields an enhancement of single-molecule photostability by greatly suppressing photobleaching pathways which involve photoexcitation from the triplet state. This is accomplished by dynamically switching off the excitation laser when a quantum jump of the molecule to the triplet state is optically detected. The resulting improvement in photostability unambiguously confirms the importance of photoexcitation from the triplet state in photobleaching dynamics and will allow the investigation of new phenomena at the single-molecule level. © 2008 American Institute of Physics. [DOI: 10.1063/1.3013843]

During the last decade, optically based single-molecule detection has become a widely used technique that has revealed many phenomena hidden in ensemble-averaged experiments, ranging from single-emitter effects in quantum optics to insights in biophysics. However, photobleaching, i.e., the irreversible conversion of an optically excited organic fluorophore into a nonfluorescent entity, is a severely limiting factor in all single-molecule studies realized under ambient conditions. Many photobleaching pathways have been reported and discussed. Several of them begin with the molecule being in the metastable triplet state to which the molecule has a small probability to jump through intersystem crossing (ISC). Indeed, photostability can be greatly improved by engineering organic fluorophores with intrinsically low ISC rates or by adding triplet state quencher. Moreover, some of these pathways may involve further photoexcitation from the T1 state. In that case, photobleaching dynamics should be modified by avoiding any light excitation while the molecule is in the T1 state.

This condition can be realized through a pulsed excitation, with a pulse duration shorter than the excited-state lifetime and a repetition period longer than the triplet lifetime τ. For each excitation pulse, the molecule will undergo a single fluorescence cycle and, if ISC occurs, it will decay back to the ground state before the following excitation pulse. This strategy is routinely used in high-power dye lasers and has recently been applied to ensemble of molecules in the context of high-resolution fluorescence microscopy.

We report an experiment that transposes this strategy to the single-molecule regime, leading to a strongly reduced photobleaching rate for an individual fluorophore under ambient conditions. The scheme consists of a feedback loop on the excitation laser based on the real-time detection of quantum jumps to the triplet state T1.

Figure 1 shows the principle of the experiment. Under light irradiation, fluorescence from an organic molecule can be described using the three-level Perrin-Jablonski representation, with cycles occurring between the ground singlet state S0 of the molecule and its first excited singlet state S1. Even though the ISC from the S1 state to the first triplet state T1 is spin forbidden, spin-orbit interaction leads to a nonradiative decay of the molecule to this level with a small probability. As relaxation from T1 to S0 is also spin forbidden, the triplet-state lifetime τT is orders of magnitude larger than the one associated with fluorescence. The experimental setup is based on a confocal microscope working under ambient conditions. Samples are made by spin coating on a glass coverslip a solution of polymethyl methacrylate (PMMA) (1% mass in anisole) doped with dye molecules at nanomolar concentration. The result is a few tens of nanometer thick polymer coating, in which molecules are well separated and can be individually probed.

![Figure 1](Image)

**FIG. 1.** (Color online) Principle of the experiment. (a) Single-molecule energy levels. (b) Photons emitted through the S1 → S0 transition are detected with a confocal microscope. The single-molecule fluorescence time trace reveals dark periods with durations on the order of the triplet-state lifetime τT corresponding to quantum jumps to the triplet state by ISC. The displayed single-molecule fluorescence signal represents the output of the photon-counting detector (D) without any binning (red bars). (c) The quantum-jump detection triggers an acousto-optical modulator (AOM), used as an on-off switch for the excitation laser. If no photon is detected during a time window of duration τD, the laser is switched off for a duration τoff longer than τT. The curve in green shows the AOM command for the fluorescence time trace represented in (b). The characteristic response time of the AOM is 600 ns, much shorter than all other time constants of the experiment.

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Fluorescence from a single molecule is detected by an avalanche photodiode operated in the photon-counting regime [Fig. 1(b)]. Due to the long lifetime of the triplet state, a nonradiative decay from $S_1$ to $T_1$ appears as a sudden drop in the fluorescence signal, corresponding to a quantum jump in the single-molecule emission time trace [16,17] [Fig. 1(b)]. The fluorescence signal is fed into an electronic card that commands in real time an acousto-optical modulator (AOM) to switch off the excitation laser when a quantum jump is detected [Fig. 1(c)]. The result is a feedback loop that performs an adaptation of light excitation to the single-molecule dynamics with two adjustable time constants, $\tau_d$ and $\tau_{off}$. As described in Fig. 1(c), $\tau_d$ is the time constant over which the decision is taken that a molecule has indeed experienced a $S_1 \rightarrow T_1$ quantum jump. This parameter acts as a temporal threshold that discriminates between fluorescence cycles and a quantum jump: if no photon is detected during $\tau_d$, the laser is switched off for a duration $\tau_{off}$ which is set to be greater than the triplet lifetime $\tau_T$.

The experiment is first performed with carbocyanine DiI. Single molecules are excited with a cw laser at 532 nm with an intensity of 1 kW cm$^{-2}$, close to the saturation of the $S_0 \rightarrow S_1$ transition. This leads to detection counting rates ranging from 30 to 200 kcounts s$^{-1}$, depending on the environment and the molecule dipole orientation. To limit the unavoidable bias which appears in single-molecule statistics measurements, we decide to study all fluorophores with avoidable bias which appears in single-molecule statistics measurement and the molecule dipole orientation. To limit the un

feedback loop is a reduction in this illumination duration from $\tau_t$ to $\tau_d$ [Fig. 1(c)], leading to a photostability enhancement factor $G$ of

$$G = \frac{\langle \tau_t \rangle}{\tau_d},$$

where $\langle \tau_t \rangle$ is the average value of the triplet-state lifetime over a set of molecules in the sample.

To test our assumptions, the experiment is performed for different values of $\tau_d$. For each set of molecules, triplet-state lifetimes are distributed with complex statistics, and will then correspond to different photostability gains. Therefore, for each value of $\tau_T$ we choose to estimate $G$ as the ratio of the medians of the photocount distributions measured with and without the feedback loop. The median is known as a robust estimator of the central value of the distribution of a random variable, even for heavy-tailed statistics that have been found in photobleaching ensemble measurements.

The experimental results show an increase in the $G$ factor as $\tau_d$ is decreased [Fig. 2(c)]. Equation (1) matches the data well for $\langle \tau_t \rangle = 240 \mu$s, consistent with previous measurements. This result provides a direct experimental verdict on the influence of triplet-state photoexcitation on photobleaching dynamics.

It is well known that photobleaching also strongly depends on the ambient atmosphere, especially the presence of oxygen. However, the effect of oxygen changes depending on the molecule. Whereas oxygen acts as a quencher of the triplet state for DiI, leading to an increase in the survival time before photobleaching, it enhances photodestruction in
the case of aromatic hydrocarbon dyes such as terrylene.\(^{22,23}\)

To investigate a potential generality of the quantum-jump-based feedback method, the experiment is reproduced with terrylene molecules. An enhancement of both the total number of emitted photons and the single-molecule survival time is again achieved using the feedback loop (Fig. 3). A photostability enhancement \(G=3.1\) is reached, consistent with the ratio \(\langle \tau_d \rangle / \tau_d\).

We have shown that a simple adaptive light excitation scheme, relying on the real-time detection of single-molecule quantum jumps, leads to an enhancement of the photostability of different types of organic fluorophores that all suffer from photobleaching. This optical strategy can be operated at room temperature and under ambient atmosphere. The observed gain in photostability highlights the importance of photoexcitation from the triplet state in photobleaching dynamics. Further photostability improvement is expected by decreasing the threshold parameter \(\tau_d\) in the feedback loop. This requires an increase in the detection collection efficiency or a decrease in the fluorescence lifetime, as can be achieved by coupling the fluorophore to a metallic surface\(^{24}\) or to a nanostructured substrate.\(^{25}\)

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\(^{18}\)For each molecule in the set of data, the survival time before photobleaching is corrected for all periods when the laser is switched off.


